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(57) Abstract

A reshaped human antibody or reshaped human antibody fragment having specificity for human placental alkaline phosphatase (PLAP) is produced by transferring the complementarity determining regions (CDRs) from a murine anti-PLAP hybridoma cell line H17E2 into a human antibody variable region framework. The reshaped molecule can be used in the treatment or diagnosis of cancer.

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SPECIFIC BINDING AGENTS

This invention relates to specific binding agents, and in particular to polypeptides containing amino acid sequences that bind specifically to other proteinaceous or non-proteinaceous materials. The invention most particularly concerns the production of such specific binding agents by genetic engineering.

Antibody structure

Natural antibody molecules consist of two identical heavy-chain and two identical light-chain polypeptides, which are covalently linked by disulphide bonds. Figure 13 of the accompanying drawings diagramatically represents the typical structure of an antibody of the IgG class. Each of the chains is folded into several discrete domains. The N-terminal domains of all the chains are variable in sequence and therefore called the variable regions (V-regions). The V-regions of one heavy (VH) and one light chain (VL) associate to form the antigen-binding site. The module formed by the combined VH and VL domains is referred to as the Fv (variable fragment) of the

antibody. The C-terminal ends of both heavy and light chains are more conserved in sequence and chains are more conserved in sequence and therefore referred to as the constant regions. Heavy chain constant regions are composed of several domains, eg. the heavy chain of the gamma-isotype (IgG) consists of three domains (CH1, CH2, CH3) and a hinge region which connects the CH1 and CH2 domains. The hinges of the two heavy chains are covalently linked together by disulphide bridges. Light chains have one constant domain which packs against the CH1 domain. The constant regions of the antibody molecule are involved in effector functions such as complement lysis and clearing by Antibody Dependant Cell Cytotoxicity (ADCC). Classical digestion of an antibody with the protease papain yields three fragments. One fragment contains the CH2 and CH3 domains and, as it crystallises easily, was called the Fc fragment. The other two fragments were designated the Fab (antigen-binding) fragments, they are identical and contain the entire light chain combined with the VH and CH1 domain. When using pepsin, the proteolytic cleavage is such that the two Fabs remain connected via the hinge and form the $(Fab)_2$ fragment. Each of the domains is represented by a separate exon at the genetic level.

The variable regions themselves each contain 3 clusters of hypervariable residues, in a framework of more conserved sequences. These hypervariable regions interact with the antigen, and are called the Complementarity Determining Regions (CDRs). The more conserved sequences are called the Framework Regions (FRs). See Kabat et al (1987). X-ray studies of antibodies have shown that the CDRs form loops which protrude from the top of the molecule, whilst the FRs provide a structural beta-sheet framework.

Modified antibodies

In one embodiment, the invention relates to so-called "reshaped" or "altered" human antibodies, ie. immunoglobulins having essentially human constant and framework regions but in which the complementarity determining regions (CDRs) correspond to those found in a non-human immunoglobulin, and also to corresponding reshaped antibody fragments.

The general principles by which such reshaped human antibodies and fragments may be produced are now well-known, and reference can be made to Jones et al (1986), Riechmann et al (1988), Verhoeyen et al (1988), and EP-A-239400 (Winter). A comprehensive list of relevant literature references is provided later in this specification.

Reshaped human antibodies and fragments have particular utility in the in-vivo diagnosis and treatment of human ailments because the essentially human proteins are less likely to induce undesirable adverse reactions when they are administered to a human patient, and the desired specificity conferred by the CDRs can be raised in a host animal, such as a mouse, from which antibodies of selected specificity can be obtained more readily. The variable region genes can be cloned from the non-human antibody, and the CDRs grafted into a human variable-region framework by genetic engineering techniques to provide the reshaped human antibody or fragment. To achieve this desirable result, it is necessary to identify and sequence at least the CDRs in the selected non-human antibody, and preferably the whole non-human variable region sequence, to allow

identification of potentially important CDR-framework interactions.

Summary of the invention

The invention provides, as one embodiment, a synthetic specific binding polypeptide having specificity for human placental alkaline phosphatase (PLAP). By synthetic, we particularly mean that the polypeptide is produced by recombinant DNA technology, and to that extent at least is different from a naturally-occurring or naturally-induced specific binding agent having identical specificity. Alternatively, the synthetic polypeptide has been produced by artificially assembling a sequence of amino acids to produce a novel or nature-identical molecule. The synthetic polypeptide can be equivalent to an intact conventional antibody, or equivalent to a multiple or single-chain fragment of such an antibody, or can be simply a material that includes one or more sequences that confer the desired specific binding capability.

The invention provides as an important embodiment, a reshaped human antibody, or a reshaped human antibody fragment, having anti-human placental alkaline phosphatase (PLAP) specificity.

More particularly, the invention provides a reshaped human antibody or reshaped human antibody fragment, having anti-human placental alkaline phosphatase specificity, containing one or more of the CDRs depicted in Figures 1 and 2 of the accompanying drawings. Preferably, the reshaped antibody or fragment of the invention contains all 3 of the CDRs depicted in Figure 1 of the accompanying drawings, in a human heavy chain variable region

framework. Alternatively, or in addition, the reshaped antibody or fragment of the invention contains all 3 of the CDRs depicted in Figure 2 of the accompanying drawings, in a human light chain variable region framework.

Another embodiment of the invention is a reshaped antibody or reshaped antibody fragment containing a protein sequence as depicted in Figure 10 and/or Figure 11 of the accompanying drawings.

Other important embodiments of the invention are an expression vector incorporating a DNA sequence as depicted in Figure 10 and/or Figure 11 of the accompanying drawings, and an expression vector incorporating a DNA sequence encoding one or more of the protein sequences designated as being a CDR in Figure 1 and/or Figure 2 of the accompanying drawings.

An important aspect of the invention is a stable host cell line containing a foreign gene that causes the host cell line to produce a specific binding agent according to the invention. This can be a stable host cell line containing a foreign gene that encodes at least one of the amino acid sequences designated as being a CDR in Figure 1 and/or Figure 2 of the accompanying drawings, together with a protein framework that enables the encoded amino acid sequence when expressed to function as a CDR having specificity for PLAP.

The invention particularly provides an immortalised mammalian cell line, or a yeast, or other eukaryotic cell, or a prokaryotic cell such as a bacterium, producing a reshaped antibody or fragment according to the invention.

Another important aspect of the invention is a synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment, having specificity equivalent to that of the gamma-1, kappa anti-PLAP monoclonal antibody secreted by murine hybridoma cell line H17E2.

The invention also provides two novel plasmids, pSVgptHu2VHPLAP-HuIgG1 and pSVneoHuVkPLAP-HuCk, and these plasmids can be used in the production of a synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment.

These plasmids are contained in novel E.coli strains NCTC 12389 and NCTC 12390, respectively.

Other aspects of the invention are:

a) A DNA sequence encoding a reshaped human antibody heavy-chain variable region having specificity for human placental alkaline phosphatase, as contained in E.coli NCTC 12389.

b) A DNA sequence encoding a reshaped human antibody light-chain variable region having specificity for human placental alkaline phosphatase, as contained in E.coli NCTC 12390.

c) A reshaped human antibody heavy-chain variable region having specificity for human placental alkaline phosphatase, producible by means of the expression vector contained in E.coli NCTC 12389.

d) A reshaped human antibody light-chain variable region having specificity for human placental alkaline

phosphatase, producible by means of the expression vector contained in E.coli NCTC 12390.

e) A reshaped human antibody or reshaped human antibody fragment, comprising at least one variable region according to c) or d) above.

A particular embodiment of the invention is therefore a reshaped human antibody or fragment possessing anti-PLAP specificity and incorporating a combination of CDRs (which may, for example, be cloned from a murine anti-PLAP immunoglobulin) having the amino acid sequences identified as CDR1, CDR2 and CDR3 respectively in Figures 1 and 2 of the accompanying drawings, which respectively represent the heavy chain variable region (VH) and light chain variable region (Vk) of a murine anti-PLAP monoclonal antibody that we have cloned and sequenced. In the case of an intact antibody, or a fragment comprising at least one heavy chain variable region and at least one light chain variable region, the reshaped antibody or fragment preferably contains all six CDRs from the non-human source. To be most effective in binding, the CDRs should preferably be sited relative to one another in the same arrangement as occurs in the original non-human antibody, e.g. the VH CDRs should be in a human VH framework, and in the order in which they occur naturally in the non-human antibody.

As will be apparent to those skilled in the art, the CDR sequences and the surrounding framework sequences can be subject to minor modifications and variations without the essential specific binding capability being significantly reduced. Such minor modifications and variations can be present either at the genetic level or in the amino acid sequence, or both. Accordingly, the

invention encompasses synthetic (reshaped) antibodies and fragments that are functionally equivalent to those described herein having precisely defined genetic or amino acid sequences.

The invention can also be applied in the production of bi-specific antibodies, having two Fab portions of different specificity, wherein one of the specificities is conferred by a reshaped human variable chain region incorporating one or more of the CDRs depicted in Figures 1 and 2 of the accompanying drawings.

The invention can also be applied in the production of so-called single-chain antibodies (for example, as disclosed in Genex EP-A-281604), and also to polysaccharide-linked antibodies (see Hybritech EP-A-315456) and other modified antibodies.

Any human constant regions (for example, gamma 1, 2, 3 or 4-type) can be used.

Antibody fragments retaining useful specific binding properties can be $(Fab)_2$, Fab, Fv, VH or Vk fragments. These can be derived from an intact reshaped antibody, for example by protease digestion, or produced as such by genetic engineering.

Practical applications of the invention

An important aspect of the invention is a reshaped human anti-PLAP antibody or fragment, as defined above linked to or incorporating an agent capable of retarding or terminating the growth of cancerous cells, or to an imaging agent capable of being detected while inside the human body. The invention also includes injectable

compositions comprising either of such combinations in a pharmaceutically acceptable carrier, such as saline solution, plasma extender or liposomes. The invention also includes the use, in a method of human cancer therapy or imaging, of a reshaped human anti-PLAP antibody or fragment as defined above. The invention further includes the use of such an antibody or fragment for the manufacture of a medicament for therapeutic application in the relief of cancer in humans, or the use of such an antibody or fragment in the manufacture of a diagnostic composition for in-vivo diagnostic application in humans.

The Fc region of the antibody, itself using pathways and mechanisms available in the body, such as complement lysis and antibody dependent cellular cytotoxicity, can be used to affect adversely the growth of cancerous cells. In this embodiment, no additional reagent need be linked to the reshaped antibody.

Examples of agents capable of affecting adversely the growth of cancerous cells include radioisotopes, such as Yttrium 90 and Iodine 131; drugs such as methotrexate; toxins such as ricin or parts thereof; and enzymes which may for example turn an inactive drug into an active drug at the site of antibody binding.

Examples of imaging agents include radioisotopes generating gamma rays, such as Indium 111 and Technetium 99; radioisotopes generating positrons, such as Copper 64; and passive agents such as Barium which act as contrast agents for X-rays, and Gadolinium in nmr/esr scanning.

In order to link a metallic agent, such as a radioisotope, to a specific binding agent of the invention, it may be necessary to employ a coupling or

chelating agent. Many suitable chelating agents have been developed, and reference can be made for example to US 4824986, US 4831175, US 4923985 and US 4622420. Techniques involving the use of chelating agents are described, for example, in US 4454106, US 4722892, Moi et al (1988), McCall et al (1990), Deshpande et al (1990) and Meares et al (1990).

The use of radiolabelled antibodies and fragments in cancer imaging and therapy in humans is described for example in EP 35265. It may be advantageous to use the radiolabelled cancer-specific antibody or fragment in conjunction with a non-specific agent radiolabelled with a different isotope, to provide a contrasting background for so-called subtraction imaging.

The antibody reagents of the invention can be used to identify, e.g. by serum testing or imaging, and/or to treat, PLAP-producing cancers. Such cancers can occur as, for example, breast cancer, ovarian cancer and colon cancer, or can manifest themselves as liquids such as pleural effusions.

Modified antibody production

The portions of the VH and VL regions that by convention (Kabat, 1987) are designated as being the CDRs may not be the sole features that need to be transferred from the non-human monoclonal antibody. Sometimes, enhanced antibody performance, in terms of specificity and/or affinity, can be obtained in the reshaped human antibody if certain non-human framework sequences are conserved in the reshaped human antibody. The objective is to conserve the important three-dimensional protein

structure associated with the CDRs, which is supported by contacts with framework residues.

The normal starting point from which a reshaped antibody in accordance with the invention can be prepared, is a cell (preferably an immortalised cell line), derived from a non-human host animal (for example, a mouse), which expresses an antibody having specificity against human PLAP. Such a cell line can, for example, be a hybridoma cell line prepared by conventional monoclonal antibody technology. Preferably, the expressed antibody has a high affinity and high specificity for PLAP, because it should be anticipated that some loss of affinity and/or specificity may occur during the transfer of these properties to a human antibody or fragment by the procedures of the invention. By selecting a high affinity and high specificity antibody as the parent antibody, the likelihood that the final reshaped antibody or fragment will also exhibit effective binding properties is enhanced.

The next stage is the cloning of the cDNA from the cell expressing the selected non-human antibody, and sequencing and identification of the variable region genes including the sequences encoding the CDRs. The procedures involved can now be regarded as routine in the art, although they are still laborious.

If the object is to produce a reshaped complete human antibody, or at least a fragment of such an antibody which will contain both heavy and light variable domains, it will be necessary to sequence the cDNA associated with both of these domains.

Once the relevant cDNA sequence or sequences have been analysed, it is necessary to prepare one or more replicable expression vectors containing a DNA sequence which encodes at least a variable domain of an antibody, which variable domain comprises human framework regions together with one or more CDRs derived from the selected non-human anti-PLAP antibody. The DNA sequence in each vector should include appropriate regulatory sequences necessary to ensure efficient transcription and translation of the gene, particularly a promoter and leader sequence operably linked to the variable domain sequence. In a typical procedure to produce a reshaped antibody or fragment in accordance with the invention, it may be necessary to produce two such expression vectors, one containing a DNA sequence for a reshaped human light chain and the other, a DNA sequence for a reshaped human heavy chain. The expression vectors should be capable of transforming a chosen cell line in which the production of the reshaped antibody or fragment will occur. Such a cell line may be, for example, a stable non-producing myeloma cell line, examples (such as NSO and sp2-0) of which are readily available commercially. An alternative is to use a bacterial system, such as E.coli, as the expression vehicle for the reshaped antibody or fragment. The final stages of the procedure therefore involve transforming the chosen cell line or organism using the expression vector or vectors, and thereafter culturing the transformed cell line or organism to yield the reshaped human antibody or fragment.

By way of example only, detailed steps by means of which appropriate expression vectors can be prepared are given later in this specification. The manipulation of DNA material in a suitably equipped laboratory is now a well-developed art, and the procedures required are well

within the skill of those versed in this art. Many appropriate genomic and cDNA libraries, plasmids, restriction enzymes, and the various reagents and media which are required in order to perform such manipulations, are available commercially from suppliers of laboratory materials. For example, genomic and cDNA libraries can be purchased from Clontech Laboratories Inc. The steps given by way of example below are purely for the guidance of the reader of this specification, and the invention is in no way critically dependant upon the availability of one or more special starting materials. In practice, the skilled person has a wide range of materials from which to choose, and can exploit and adapt the published technology using acquired experience and materials that are most readily available in the scientific environment. For example, many plasmids fall into this category, having been so widely used and circulated within the relevant scientific community that they can now be regarded as common-place materials.

Examples

The procedure used to prepare reshaped anti-PLAP human antibodies is described in detail below, by way of example only, with reference to the accompanying drawings, of which:

Figure 1 shows the cDNA sequence coding for a murine heavy chain variable region having anti-PLAP specificity. The 3 classical CDRs are indicated, together with an amino acid sequence matching the cDNA code.

Figure 2 shows the cDNA sequence coding for a murine light chain variable region having anti-PLAP specificity.

Figures 3a and 3b together show a route by which an expression vector encoding a reshaped human heavy chain incorporating the CDRs of Figure 1, can be prepared.

Figures 4a and 4b together show a similar transformation route to obtain an expression vector encoding a reshaped human light chain incorporating the CDRs of Figure 2, can be prepared.

Figure 5 shows the plasmid pU12-IgEnh, which contains an enhancer sequence used in the routes of Figures 4a and 4b.

Figure 6 shows the source of plasmid pBGS18-HulgG1 used in the route of Figure 3b.

Figure 7 shows the source of plasmid pBGS18-HuCk used in the route of Figure 4b.

Figure 8 shows two synthetic oligonucleotide sequences I and II used in cloning the cDNA sequences of Figures 1 and 2.

Figure 9 shows six synthetic oligonucleotide sequences III to VIII used in the routes depicted in Figures 3a-4b.

Figures 10 and 11 show the cDNA and amino acid sequences of the resulting reshaped human heavy and light chain variable regions respectively.

Figure 12 shows in graphical form the relative specific anti-PLAP binding activity of the resulting reshaped human antibody.

Figure 13 depicts in diagrammatic form the structure of a typical antibody (immunoglobulin) molecule.

The experimental procedures required to practice the invention do not in themselves represent unusual technology, and they involve straightforward cloning and mutagenesis techniques as generally described for example in Verhoeyen et al (1988); Riechmann et al (1988) and EP-A-239400 (Winter). Alternatively, if an appropriate DNA sequence is already known in detail (the drawings accompanying this specification includes a sequence associated with anti-PLAP specificity), the reshaped human variable region genes can be synthesised in vitro (see Jones et al, 1986). Laboratory equipment and reagents for synthesising long oligonucleotides are readily available, and as techniques in this field develop it is becoming practicable to synthesise progressively longer sequences.

Detailed laboratory manuals, covering all basic aspects of recombinant DNA techniques, are available, e.g. "Molecular Cloning" by Sambrook et al (1989).

By means of the invention, the antigen binding regions of a mouse anti-PLAP antibody were grafted onto human framework regions. The resulting reshaped human antibody (designated Hu2PLAP) has binding characteristics similar to those of the original mouse antibody.

Such reshaped antibodies can be used for in vivo diagnosis and treatment of human cancers, eg. ovarian cancers and seminoma, and are expected at least to reduce the problem of an immune response in the patient often seen upon administration of non-human antibody. A similar benefit has been shown for reshaped CAMPATH-1 antibody in Hale et al (1988).

Methods:

a) Cloning and sequence determination of the mouse variable region genes

Messenger RNA was isolated from the murine hybridoma line "H17E2" which secretes a gamma-1, kappa anti-PLAP antibody, described in Travers et al (1984). First strand cDNA was synthesised by priming with oligonucleotides I and II (see Figure 8) complementary to the 5' ends of the CH1 and Ck exons respectively. Second strand cDNA was obtained as described by Gübler and Hoffmann (1983).

Kinased EcoRI linkers were ligated to the now double-stranded cDNA (which was first treated with EcoRI methylase, to protect possible internal EcoRI sites), followed by cloning into EcoRI-cut pUC9 (Vieira et al, 1982) and transformation of E.coli strain TG2 (Gibson, 1984).

Colonies containing genes coding for murine anti-PLAP VH (MoVHPLAP) and for murine anti-PLAP Vk (MoVkPLAP) were identified by colony hybridisation with 2 probes consisting respectively of 32P-labelled first strand cDNA of anti-PLAP VH and Vk. Positive clones were characterised by plasmid preparation, followed by EcoRI digestion and 1.5% agarose gel analysis. Full-size inserts (about 450bp) were subcloned in the EcoRI site of M13mp18 (Norrander et al, 1983). This yielded clones with inserts in both orientations, facilitating nucleotide sequence determination of the entire insert, by the dideoxy chain termination method (Sanger et al, 1977).

The nucleotide sequences, and their translation into amino acid sequences, of the mature variable region genes

MoVHPLAP and MoVkPLAP, are shown in Figures 1 and 2. The 450 bp inserts included a signal sequence and 5' untranslated sequences and linkers, not shown in the Figures.

b) Grafting of the mouse anti-PLAP CDRs onto human framework regions

The general techniques necessary to achieve this have been described very adequately in Jones et al (1986), Verhoeyen et al (1988), Riechmann et al (1988) and in EP-A-239400 (Winter).

The basic constructs used for reshaping were M13mp9HuVHLYS (Verhoeyen et al, 1988) and M13mp9HuVkLYS (Riechmann et al, 1988), which respectively contain the framework regions of the heavy chain variable region of human "NEW" and of the light chain variable region of human "REI". Both of these human antibodies have been thoroughly characterised and reported (Saul et al, 1978; and Epp et al, 1974, respectively).

The CDRs in these constructs (Figures 3a and 4a) were replaced by site-directed mutagenesis with oligonucleotides encoding the anti-PLAP CDRs flanked by at least 12 nucleotides at each end encoding the corresponding human framework residues. These oligonucleotides are shown in Figure 9, in which the sequences corresponding to the CDRs are underlined.

In the present instance we found it useful also to conserve the amino acids Phe 27 and Thr 30 of the murine VHPLAP in the VH domain of the reshaped human anti-PLAP antibody. In oligonucleotide III, with 24 nucleotides

flanking the 5' end of CDR 1, the murine Phe 27 and Thr 30 codons are shown in italics in Figure 9.

The mutagenesis was done as described in Riechmann et al (1988). The resulting variable regions were named Hu2VHPLAP and HuVkPLAP and are shown in Figure 10 and 11.

c) Assembly of reshaped human antibody genes in expression vectors

The next stage involved the use of a murine heavy chain enhancer IgEnh, described in Neuberger et al (1983) where the enhancer is contained in a 1kb XbaI fragment of plasmid pSV-Vμ1. The 700bp XbaI/EcoRI subfragment of this 1kb XbaI fragment is sufficient to confer enhancer activity.

The reshaped human genes as prepared in section (b) above were excised from the M13 vectors as HindIII - BamHI fragments. The heavy chain variable region genes were cloned into a vector based on pSV2gpt (Mulligan et al, 1981), and the light chain variable region genes were cloned into a vector based on pSV2neo (Southern et al, 1981). Both contained the immunoglobulin heavy chain enhancer IgEnh. In the pSV2gpt based antibody expression vector (see Fig. 4b - 4c), the XbaI/EcoRI enhancer containing fragment was cloned in the unique EcoRI site of the pSV2gpt vector (after ligating EcoRI linkers to the filled in XbaI end of the fragment). The vector pSVgptMoVHLYS-MoIgG1 (Verhoeyen et al, 1988) was used as the source of a pSVgpt-based vector containing the IgEnh enhancer.

In the pSVneo based antibody expression vector (see Fig. 5a - 5b), the 1kb XbaI enhancer containing fragment

was first cloned into pUC12 (Vieira et al, 1982), yielding the plasmid pUC12-IgEnh, see Figure 5. The enhancer can then be cut out as a 700bp EcoRI/HindIII fragment (either orientation of the enhancer will work), and cloned in the pSV2neo-derived vector (pSVneoMSN409 as shown in Figure 4a) obtained by removing the HindIII site in pSVneo. It is possible to use pSV2gpt as an alternative vector for light chain expression, as in practice there is no need for neo selection.

The Hu2VHPLAP gene was linked to a human gamma 1 constant region (Takahashi et al, 1982), cloned initially as a 8kb HindIII fragment into the HindIII site of pBGS18 (Spratt et al, 1986), and then in the pSV2gpt expression vector as a BamHI fragment (see Figures 3b and 6). It should be noted that in the Takahashi et al (1982) reference there is an error in Figure 1: the last (3') two sites are BamH1 followed by HindIII, and not the converse. This was confirmed by Flanagan et al (1982).

The HuVkPLAP gene was linked to a human C kappa constant region (Hieter et al, 1980) also cloned in as a BamHI fragment (see Figures 4b and 7). The source of the human Ck used in Figure 7 is given in Hieter et al (1980). The 12 kb BamH1 fragment from embryonic DNA (cloned in a gamma Ch28 vector system) was subcloned in the BamH1 site of plasmid pBR322.

d) Expression in myeloma cells

Co-transfection of the expression plasmids pSVgptHu2VHPLAP-HuIgG1 and pSVneoHuVkPLAP-HuCk (Figures 3b and 4b) into NSO myeloma cells was done by electroporation (Potter et al, 1984), after linearisation with PvuI. Transfectomas were selected in mycophenolic acid

containing medium to select for cells expressing the gpt gene product, and screened for antibody production and anti-PLAP activity by ELISA assays.

Positive clones were subcloned by limiting dilution and pure clones were assayed again for anti-PLAP activity, and the best producing clones were grown in serum-free medium for antibody production.

e) <u>Binding ability of the reshaped human antibodies</u>

The practical application of the reshaped human antibody demands sufficient binding effectiveness. If the parent antibody has a very high effectiveness then some reduction during reshaping can be tolerated. The binding effectiveness will be dictated by many factors, one of which will be the antibody affinity for antigen, in this case placental alkaline phosphatase. A useful way of demonstrating binding ability of the reshaped antibody is to show that it has a similar antibody dilution curve when binding to antigen adsorbed on a plastic well surface. Such curves were generated as follows, using the parent murine anti-PLAP antibody and a reshaped human antibody prepared by the foregoing procedure.

Multiwell plates (Costar 6595, PETG) were coated with placental alkaline phosphatase (5 μg/ml in phosphate buffered saline pH 7.4, 37°C, 2 hours). The plates were rinsed in phosphate buffered saline before blocking with gelatin (0.02% in phosphate buffered saline) for one hour at room temperature, then washed four times with phosphate buffered saline with added Tween 20 (0.15%), and then used.

Antibody binding was performed in phosphate buffered saline with Tween 20 at room temperature for one hour, followed by four washes in buffer.

Visualisation of bound antibody was with horse radish peroxidase conjugated anti-globulins (anti-human IgG for the reshaped antibody and anti-mouse IgG for the parent molecule). The conjugate (Sigma) in buffer (1:1000) was incubated for one hour at room temperature, followed by four washes as above. Colour development (45 minutes) was with tetramethyl benzidene (0.01%) and hydrogen peroxide (1:200 or 100 vols) in citrate buffer pH6.5. The reaction was stopped with 2M hydrochloric acid.

Controls showed insignificant colour due to non-specific binding of conjugate or due to binding of antibody to wells not containing placental alkaline phosphatase. The results, shown in Figure 12, are expressed as a percentage of the maximum colour (binding) seen. The two curves are similar, indicating a significant and useful level of binding effectiveness for the reshaped antibody of the invention.

f) <u>Deposited plasmids</u>

<u>E.coli</u> strains containing plasmids used in the above procedure have been deposited, in accordance with the provisions of the Budapest Treaty, in the National Collection of Type Cultures on 19 April 1990 as follows:

NCTC 12389: K12, TG1 <u>E.coli</u> containing plasmid pSVgptHu2VHPLAP-HuIgG1

NCTC 12390: K12, TG1 <u>E.coli</u> containing plasmid pSVneoHuVkPLAP-HuCk

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CLAIMS

1. A synthetic specific binding agent having specificity for human placental alkaline phosphatase.

2. A synthetic specific binding agent according to claim 1, wherein the anti-PLAP specificity is conferred by the presence of one or more of the amino acid sequences:

i) Ser Tyr Gly Val Ser

ii) Val Ile Trp Glu Asp Gly Ser Thr Asn Tyr His Ser Ala Leu Ile Ser

iii) Pro His Tyr Gly Ser Ser Tyr Val Gly Ala Met Glu Tyr

iv) Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Val Ala

v) Asn Ala Lys Ser Leu Ala Glu

vi) Gln His His Tyr Val Ser Pro Trp Thr

3. A reshaped human antibody, or a reshaped human antibody fragment, having specificity for human placental alkaline phosphatase.

4. A reshaped human antibody or reshaped human antibody fragment according to claim 3, wherein the anti-PLAP specificity is conferred by the presence of one or more of the amino acid sequences:

i) Ser Tyr Gly Val Ser

ii) Val Ile Trp Glu Asp Gly Ser Thr Asn Tyr His Ser Ala Leu Ile Ser

iii) Pro His Tyr Gly Ser Ser Tyr Val Gly Ala Met Glu Tyr

iv) Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Val Ala

v) Asn Ala Lys Ser Leu Ala Glu

vi) Gln His His Tyr Val Ser Pro Trp Thr

5. A reshaped human antibody or reshaped human antibody fragment according to claim 3, having at least one heavy-chain variable region incorporating the following CDRs:

CDR1: Ser Tyr Glu Val Ser

CDR2: Val Ile Trp Glu Asp Gly Ser Thr Asn Tyr His Ser Ala Leu Ile Ser

CDR3: Pro His Tyr Gly Ser Ser Tyr Val Gly Ala Met Glu Tyr

6. A reshaped human antibody or reshaped human antibody fragment according to claim 3, having at least one light-chain variable region incorporating the following CDRs:

CDR1: Arg Ala Ser Gly Asn Ile Tyr Ser Tyr Val Ala

CDR2: Asn Ala Lys Ser Leu Ala Glu

CDR3: Gln His His Tyr Val Ser Pro Trp Thr

7. A reshaped human antibody or reshaped human antibody fragment according to claim 3 and having at least one heavy-chain variable region according to claim 5 and at

least one light-chain variable region according to claim 6.

8. A reshaped human antibody or reshaped human antibody fragment according to claim 3, incorporating at least one heavy-chain variable region comprising the entire amino acid sequence depicted in Figure 10 of the accompanying drawings.

9. A reshaped human antibody or reshaped human antibody fragment according to claim 3, incorporating at least one light-chain variable region comprising the entire amino acid sequence depicted in Figure 11 of the accompanying drawings.

10. A synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment, having specificity equivalent to that of the gamma-1, kappa anti-PLAP monoclonal antibody secreted by murine hybridoma cell line H17E2.

11. A stable host cell line producing a synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment according to any one of claims 1 to 10, resulting from incorporation in the cell line of a foreign gene encoding the synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment.

12. A stable host cell line according to claim 11, wherein the foreign gene includes one or more of the nucleotide sequences:

i) AGT TAT GGT GTA AGC

ii) GTA ATA TGG GAA GAC GGG AGC ACA AAT TAT CAT TCA GCT CTC ATA TCC

iii) CCC CAC TAC GGT AGC AGC TAC GTG GGG GCT ATG GAA TAC

iv) CGA GCA AGT GAA AAT ATT TAC AGT TAT GTA GCA

v) AAT GCA AAA TCC TTA GCA GAG

vi) CAA CAT CAT TAT GTT AGT CCG TGG ACG

13. A stable host cell line according to claim 11, wherein the foreign gene includes the entire nucleotide sequence depicted in Figure 10 of the accompanying drawings.

14. A stable host cell line according to claim 11, wherein the foreign gene includes the entire nucleotide sequence depicted in Figure 11 of the accompanying drawings.

15. A stable host cell line according to claim 11, wherein the foreign gene encodes:

a) at least one of the amino acid sequences:

i) Ser Tyr Gly Val Ser

ii) Val Ile Trp Glu Asp Gly Ser Thr Asn Tyr His Ser Ala Leu Ile Ser

iii) Pro His Tyr Gly Ser Ser Tyr Val Gly Ala Met Glu Tyr

iv) Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Val Ala

v) Asn Ala Lys Ser Leu Ala Glu

vi) Gln His His Tyr Val Ser Pro Trp Thr

and b) a protein framework that enables the encoded amino acid sequence when expressed to function as a CDR having specificity for human placental alkaline phosphatase.

16. A stable host cell line according to claim 11, wherein the foreign gene encodes the entire amino acid sequence depicted in Figure 10 of the accompanying drawings.

17. A stable host cell line according to claim 11, wherein the foreign gene encodes the entire amino acid sequence depicted in Figure 11 of the accompanying drawings.

18. Plasmid pSVgptHu2VHPLAP-HuIgG1.

19. Plasmid pSVneoHuVkPLAP-HuCk.

20. Use of plasmid according to claim 18 or claim 19 in the production of a synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment.

21. E.coli NCTC 12389.

22. E.coli NCTC 12390.

23. A DNA sequence encoding a reshaped human antibody heavy-chain variable region having specificity for human placental alkaline phosphatase, as contained in E.coli NCTC 12389.

24. A DNA sequence encoding a reshaped human antibody light-chain variable region having specificity for human placental alkaline phosphatase, as contained in E.coli NCTC 12390.

25. A reshaped human antibody heavy-chain variable region having specificity for human placental alkaline phosphatase, producible by means of the expression vector contained in E.coli NCTC 12389.

26. A reshaped human antibody light-chain variable region having specificity for human alkaline phosphatase, producible by means of the expression vector contained in E.coli NCTC 12390.

27. A reshaped human antibody or reshaped human antibody fragment, comprising at least one variable region according to claim 25 or claim 26.

28. A synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment, according to any one of claims 1 to 10 or claim 27, linked to or incorporating an agent capable of retarding or terminating the growth of cancerous cells, or linked to an agent capable of being detected while inside the human body.

29. An injectable composition comprising a synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment, according to claim 28, in a pharmaceutically acceptable carrier.

30. Use of a synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment, according to any one of claims 1 to 10 or claim 27, for the manufacture of a medicament for therapeutic

application in the relief of cancer in humans, or for the manufacture of a diagnostic composition for in-vivo diagnostic application in humans.

31. Use of a synthetic binding agent, reshaped human antibody or reshaped human antibody fragment, according to claim 28, in a method of human cancer therapy or imaging.

AMEMDED CLAIMS

[received by the International Bureau
on 07 May 1991 (07.05.91);
original claims 1 and 2 replaced by new claim 1;
claims 3 and 4 replaced by new claim 2;
claims 5-31 unchanged but renumbered as claims 3-29 (6 pages)]

----

1. A synthetic specific binding agent having specificity for human placental alkaline phosphatase conferred by the presence of one or more of the amino acid sequences:

i) Ser Tyr Gly Val Ser

ii) Val Ile Trp Glu Asp Gly Ser Thr Asn Tyr His Ser Ala Leu Ile Ser

iii) Pro His Tyr Gly Ser Ser Tyr Val Gly Ala Met Glu Tyr

iv) Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Val Ala

v) Asn Ala Lys Ser Leu Ala Glu

vi) Gln His His Tyr Val Ser Pro Trp Thr

2. A reshaped human antibody, or a reshaped human antibody fragment, having specificity for human placental alkaline phosphatase conferred by the presence of one or more of the amino acid sequences:

i) Ser Tyr Gly Val Ser

ii) Val Ile Trp Glu Asp Gly Ser Thr Asn Tyr His Ser Ala Leu Ile Ser

iii) Pro His Tyr Gly Ser Ser Tyr Val Gly Ala Met Glu Tyr

iv) Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Val Ala

v) Asn Ala Lys Ser Leu Ala Glu

vi) Gln His His Tyr Val Ser Pro Trp Thr

3. A reshaped human antibody or reshaped human antibody fragment according to claim 2, having at least one heavy-chain variable region incorporating the following CDRs:

CDR1: Ser Tyr Gly Val Ser

CDR2: Val Ile Trp Glu Asp Gly Ser Thr Asn Tyr His Ser Ala Leu Ile Ser

CDR3: Pro His Tyr Gly Ser Ser Tyr Val Gly Ala Met Glu Tyr

4. A reshaped human antibody or reshaped human antibody fragment according to claim 2, having at least one light-chain variable region incorporating the following CDRs:

CDR1: Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Val Ala

CDR2: Asn Ala Lys Ser Leu Ala Glu

CDR3: Gln His His Tyr Val Ser Pro Trp Thr

5. A reshaped human antibody or reshaped human antibody fragment according to claim 2 and having at least one heavy-chain variable region according to claim 3 and at least one light-chain variable region according to claim 4.

6. A reshaped human antibody or reshaped human antibody fragment according to claim 2, incorporating at least one heavy-chain variable region comprising the entire amino

acid sequence depicted in Figure 10 of the accompanying drawings.

7. A reshaped human antibody or reshaped human antibody fragment according to claim 2, incorporating at least one light-chain variable region comprising the entire amino acid sequence depicted in Figure 11 of the accompanying drawings.

8. A synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment, having specificity equivalent to that of the gamma-1, kappa anti-PLAP monoclonal antibody secreted by murine hybridoma cell line H17E2.

9. A stable host cell line producing a synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment according to any one of claims 1 to 8, resulting from incorporation in the cell line of a foreign gene encoding the synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment.

10. A stable host cell line according to claim 9, wherein the foreign gene includes one or more of the nucleotide sequences:

i) AGT TAT GGT GTA AGC

ii) GTA ATA TGG GAA GAC GGG AGC ACA AAT TAT CAT TCA GCT CTC ATA TCC

iii) CCC CAC TAC GGT AGC AGC TAC GTG GGG GCT ATG GAA TAC

iv) CGA GCA AGT GAA AAT ATT TAC AGT TAT GTA GCA

v) AAT GCA AAA TCC TTA GCA GAG

vi) CAA CAT CAT TAT GTT AGT CCG TGG ACG

11. A stable host cell line according to claim 9, wherein the foreign gene includes the entire nucleotide sequence depicted in Figure 10 of the accompanying drawings.

12. A stable host cell line according to claim 9, wherein the foreign gene includes the entire nucleotide sequence depicted in Figure 11 of the accompanying drawings.

13. A stable host cell line according to claim 9, wherein the foreign gene encodes:

a) at least one of the amino acid sequences:

i) Ser Tyr Gly Val Ser

ii) Val Ile Trp Glu Asp Gly Ser Thr Asn Tyr His Ser Ala Leu Ile Ser

iii) Pro His Tyr Gly Ser Ser Tyr Val Gly Ala Met Glu Tyr

iv) Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Val Ala

v) Asn Ala Lys Ser Leu Ala Glu

vi) Gln His His Tyr Val Ser Pro Trp Thr

and b) a protein framework that enables the encoded amino acid sequence when expressed to function as a CDR having specificity for human placental alkaline phosphatase.

14. A stable host cell line according to claim 9, wherein the foreign gene encodes the entire amino acid sequence depicted in Figure 10 of the accompanying drawings.

15. A stable host cell line according to claim 9, wherein the foreign gene encodes the entire amino acid sequence depicted in Figure 11 of the accompanying drawings.

16. Plasmid pSVgptHu2VHPLAP-HuIgG1.

17. Plasmid pSVneoHuVkPLAP-HuCk.

18. Use of plasmid according to claim 16 or claim 17 in the production of a synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment.

19. E.coli NCTC 12389.

20. E.coli NCTC 12390.

21. A DNA sequence encoding a reshaped human antibody heavy-chain variable region having specificity for human placental alkaline phosphatase, as contained in E.coli NCTC 12389.

22. A DNA sequence encoding a reshaped human antibody light-chain variable region having specificity for human placental alkaline phosphatase, as contained in E.coli NCTC 12390.

23. A reshaped human antibody heavy-chain variable region having specificity for human placental alkaline phosphatase, producible by means of the expression vector contained in E.coli NCTC 12389.

24. A reshaped human antibody light-chain variable region having specificity for human alkaline phosphatase, producible by means of the expression vector contained in E.coli NCTC 12390.

25. A reshaped human antibody or reshaped human antibody fragment, comprising at least one variable region according to claim 23 or claim 24.

26. A synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment, according to any one of claims 1 to 8or claim 25, linked to or incorporating an agent capable of retarding or terminating the growth of cancerous cells, or linked to an agent capable of being detected while inside the human body.

27. An injectable composition comprising a synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment, according to claim 26, in a pharmaceutically acceptable carrier.

28. Use of a synthetic specific binding agent, reshaped human antibody or reshaped human antibody fragment, according to any one of claims 1 to 8 or claim 25, for the manufacture of a medicament for therapeutic application in the relief of cancer in humans, or for the manufacture of a diagnostic composition for in-vivo diagnostic application in humans.

29. Use of a synthetic binding agent, reshaped human antibody or reshaped human antibody fragment, according to claim 26, in a method of human cancer therapy or imaging.

Fig.1.

MoVHPLAP

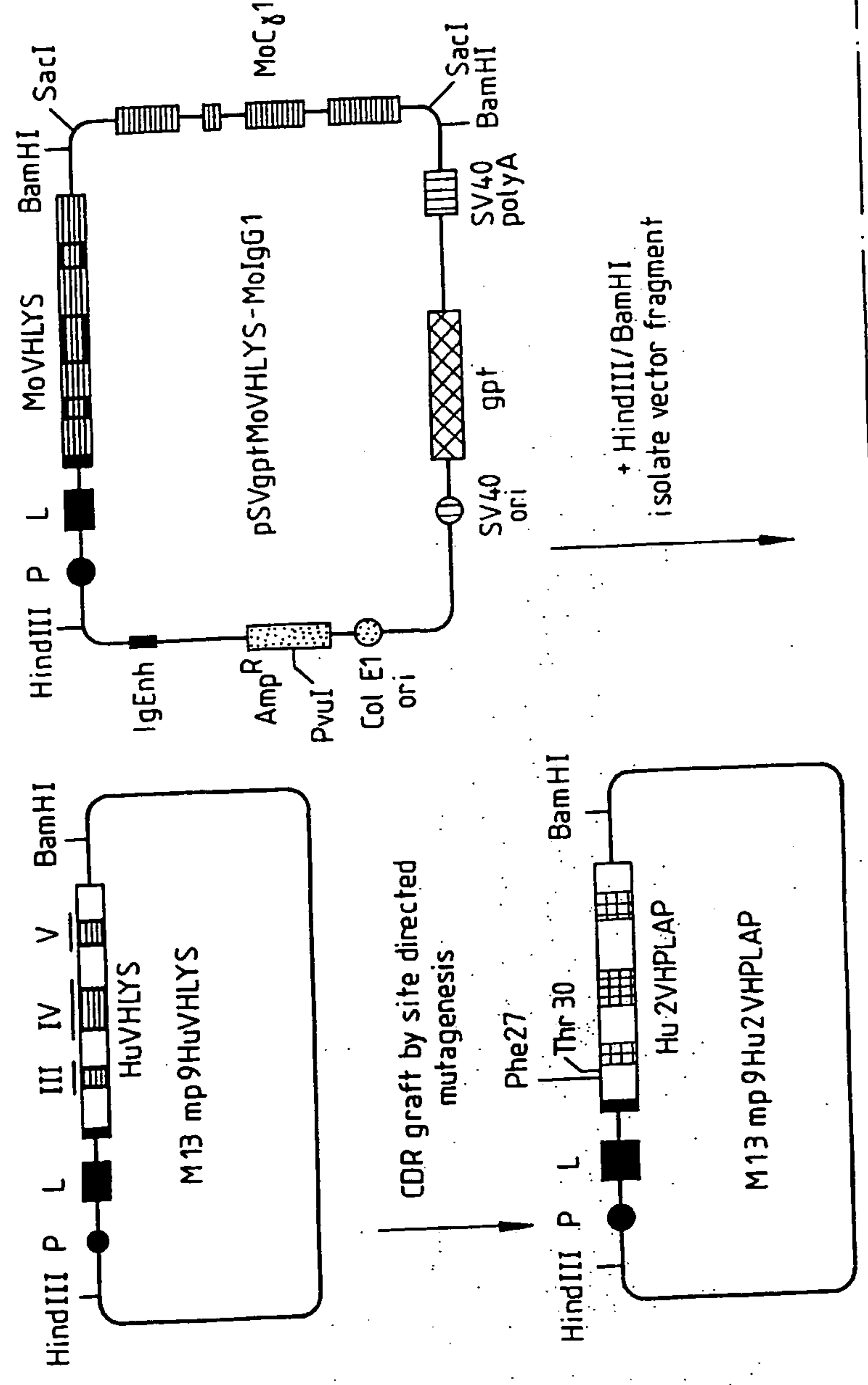
```
                5                   10                  15                  20
CAG GTG CAG CTG AAG GAG TCA GGA CCT GGC CTG GTG GCG CCC TCA CAG AGC CTG TCC ATC    60
Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Ile

                25                  30      CDR1    35                      40
ACA TGC ACT GTC TCA GGG TTC TCA TTA ACC AGT TAT GGT GTA AGC TGG GTT CGC CAG CCT   120
Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr Gly Val Ser Trp Val Arg Gln Pro

                45                      50                  55      CDR 2   60
CCA AGA AAG GGT CTG GAG TGG CTG GGA GTA ATA TGG GAA GAC GGG AGC ACA AAT TAT CAT   180
Pro Arg Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Glu Asp Gly Ser Thr Asn Tyr His

                65                      70                  75                  80
TCA GCT CTC ATA TCC AGA CTG AGC ATC AAC AAG GAT AAC TCC AAG AGC CAA GTT TTC TTA   240
Ser Ala Leu Ile Ser Arg Leu Ser Ile Asn Lys Asp Asn Ser Lys Ser Gln Val Phe Leu

    82  A   B   C   83      85                  90                  95      CDR3
AAA CTG AAC AGT CTG CAA ACT GAT GAC ACA GCC ACG TAC TAC TGT GCC AAA CCC CAC TAC   300
Lys Leu Asn Ser Leu Gln Thr Asp Asp Thr Ala Thr Tyr Tyr Cys Ala Lys Pro His Tyr

        100 A   B   C   D   E   101             105                 110
GGT AGC AGC TAC GTG GGG GCT ATG GAA TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC   360
Gly Ser Ser Tyr Val Gly Ala Met Glu Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser

TCA                                                                               363
Ser
```

Fig. 2.

MoVkPLAP

```
                 5                  10                  15                  20
GAC ATC CAG ATG ACT CAG TCT CCA GCC TCC CTA ACT GCA TCT GTG GGA GAA ACT GTC ACC    60
Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Thr Ala Ser Val Gly Glu Thr Val Thr
                25     CDR 1        30                  35                  40
ATC ACC TGT CGA GCA AGT GAA AAT ATT TAC AGT TAT GTA GCA TGG TAT CAG CAG AAA CAG   120
Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Val Ala Trp Tyr Gln Gln Lys Gln
                45                  50     CDR 2        55                  60
GGA AAA TCT CCT CAG TTC CTG GTC TAT AAT GCA AAA TCC TTA GCA GAG GGT GTG CCA TCA   180
Gly Lys Ser Pro Gln Phe Leu Val Tyr Asn Ala Lys Ser Leu Ala Glu Gly Val Pro Ser
                65                  70                  75                  80
AGG TTC AGT GGC AGT GGA TCA GGC ACA CAG TTT TCT CTG AAG ATC AAC AGC CTG CAG CCT   240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Phe Ser Leu Lys Ile Asn Ser Leu Gln Pro
                85                  90     CDR 3        95                 100
GAA GAT TTT GGG AAT TAT TAC TGT CAA CAT CAT TAT GTT AGT CCG TGG ACG TTC GGT GGA   300
Glu Asp Phe Gly Asn Tyr Tyr Cys Gln His His Tyr Val Ser Pro Trp Thr Phe Gly Gly
                105
GGC ACC AAG CTG GAA ATC AGA CGG                                                   324
Gly Thr Lys Leu Glu Ile Arg Arg
```

Fig. 3a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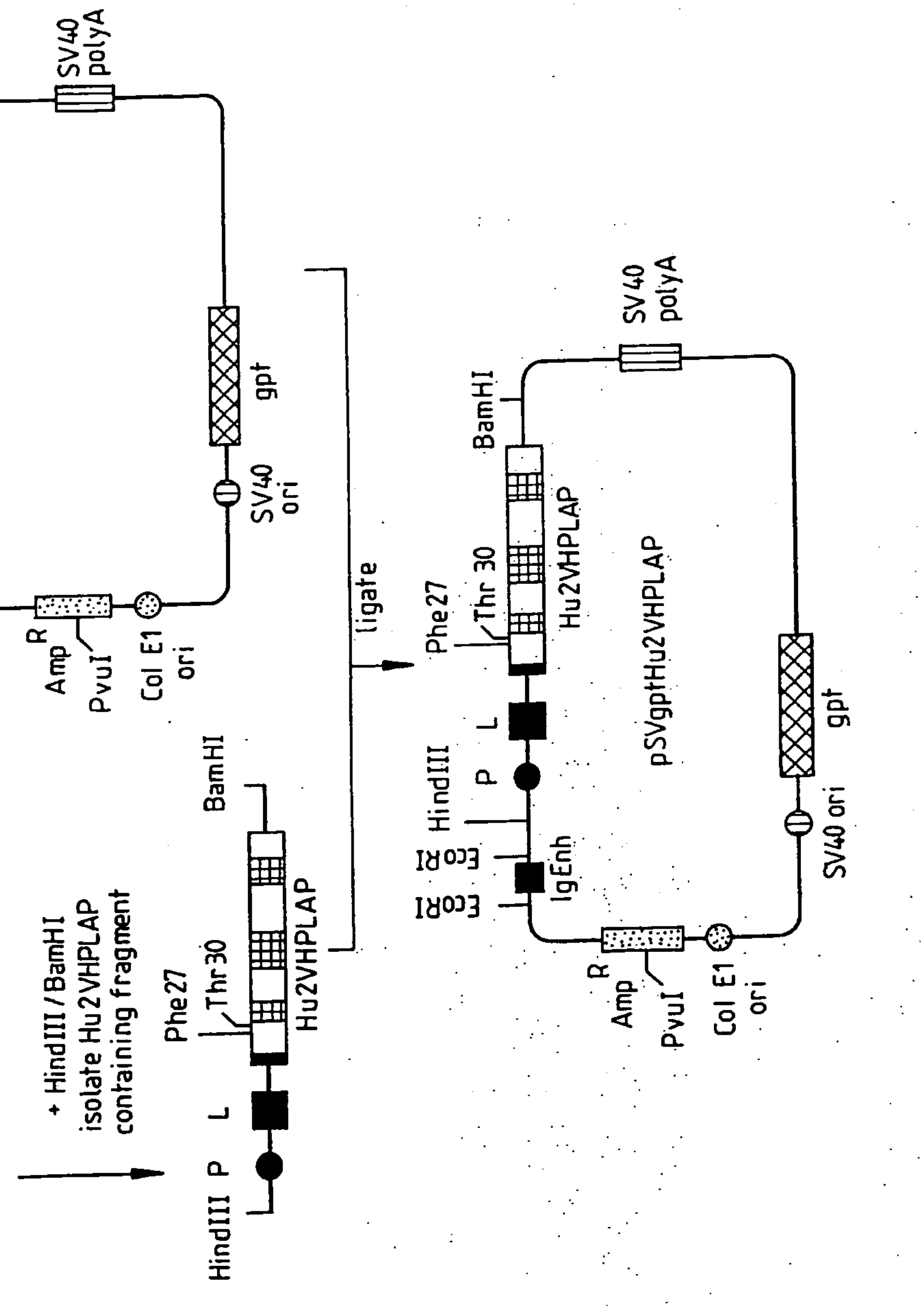
SacI
MoCγ1
SacI
BamHI
MoVHLYS
SV40
polyA
pSVgptMoVHLYS-MoIgG1
gpt
L
P
HindIII
IgEnh
Amp^R
PvuI
Col E1
ori
SV40
ori
+ HindIII/BamHI
isolate vector fragment
BamHI
V
IV
III
HuVHLYS
M13 mp9HuVHLYS
L
P
HindIII
CDR graft by site directed
mutagenesis
BamHI
Phe27
Thr 30
Hu2VHPLAP
M13 mp9Hu2VHPLAP
L
P
HindIII

Fig.3a(cont.)


SV40
polyA
BamHI
HindIII
IgEnh
gpt
SV40
ori
Amp R
PvuI
Col E1
ori
ligate
+ HindIII / BamHI
isolate Hu2VHPLAP
containing fragment
HindIII
P
L
Phe27
Thr30
Hu2VHPLAP
BamHI
pSVgptHu2VHPLAP
EcoRI
EcoRI
SV40 ori

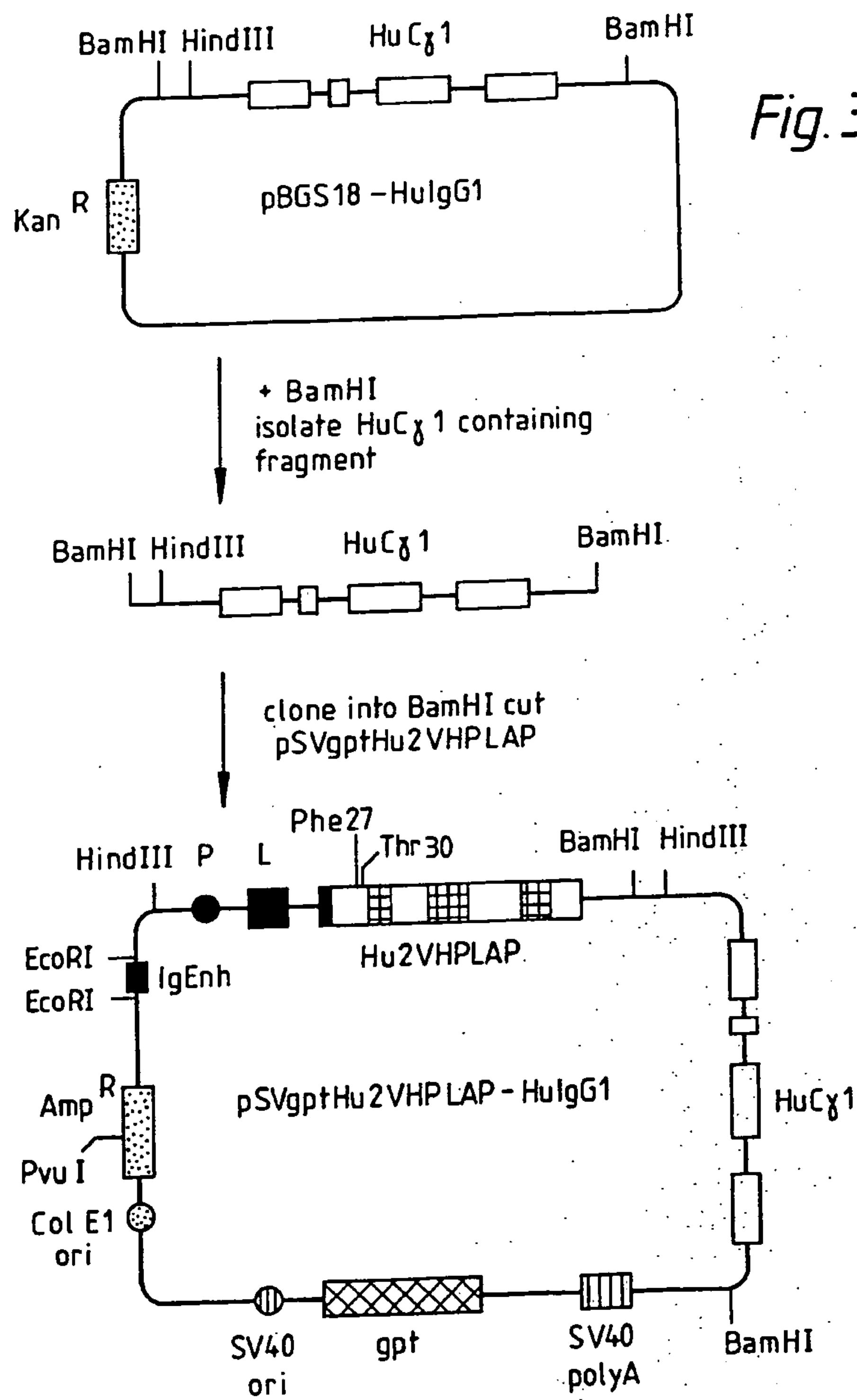

BamHI HindIII
HuCγ1
BamHI
KanR
pBGS18 –HuIgG1
+ BamHI
isolate HuCγ1 containing
fragment
BamHI HindIII
HuCγ1
BamHI
clone into BamHI cut
pSVgptHu2VHPLAP
HindIII P L
Phe27
Thr30
BamHI HindIII
Hu2VHPLAP
EcoRI
IgEnh
EcoRI
AmpR
pSVgptHu2VHPLAP - HuIgG1
HuCγ1
Pvu I
Col E1
ori
SV40
ori
gpt
SV40
polyA
BamHI

Fig. 3b

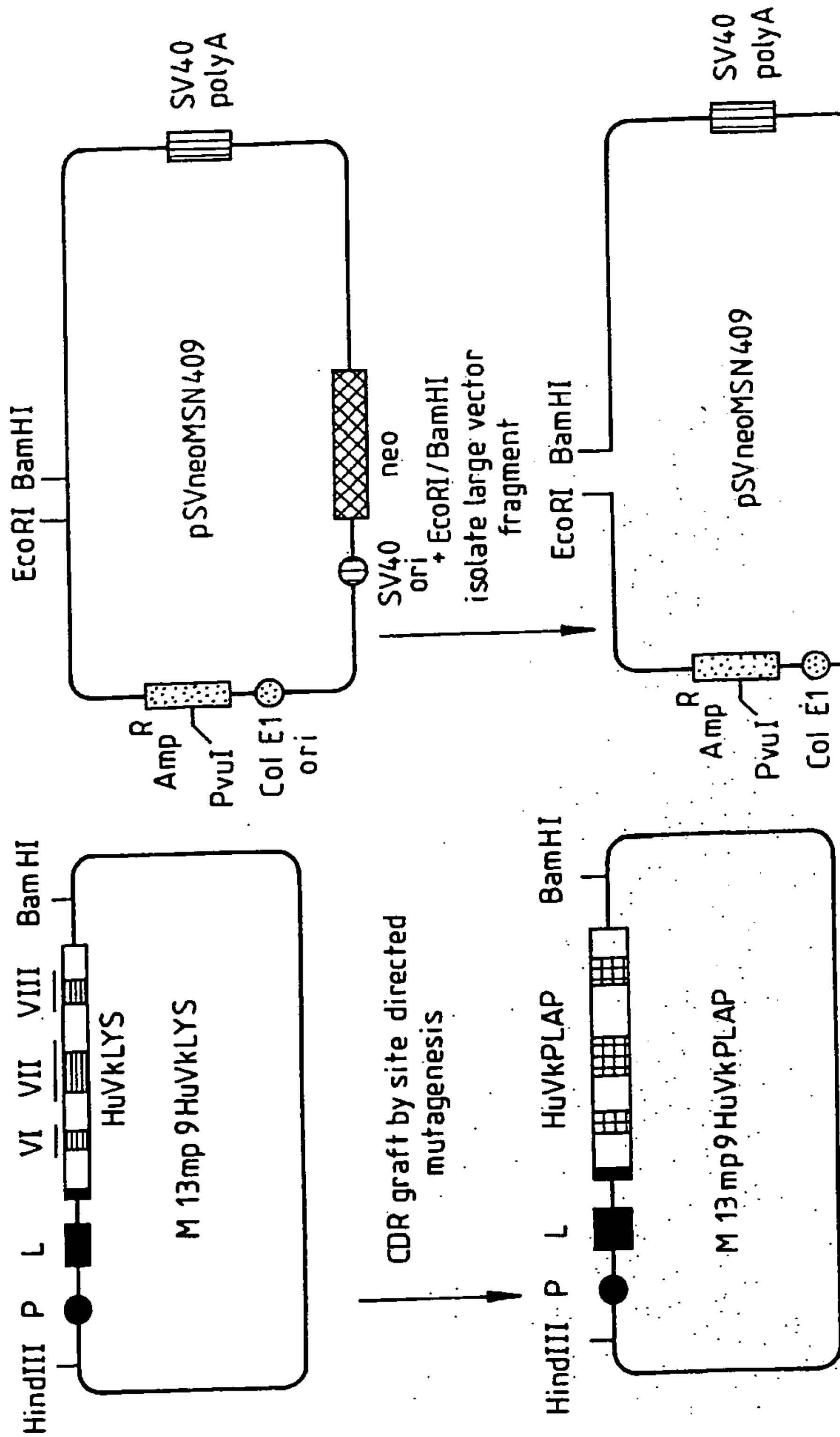
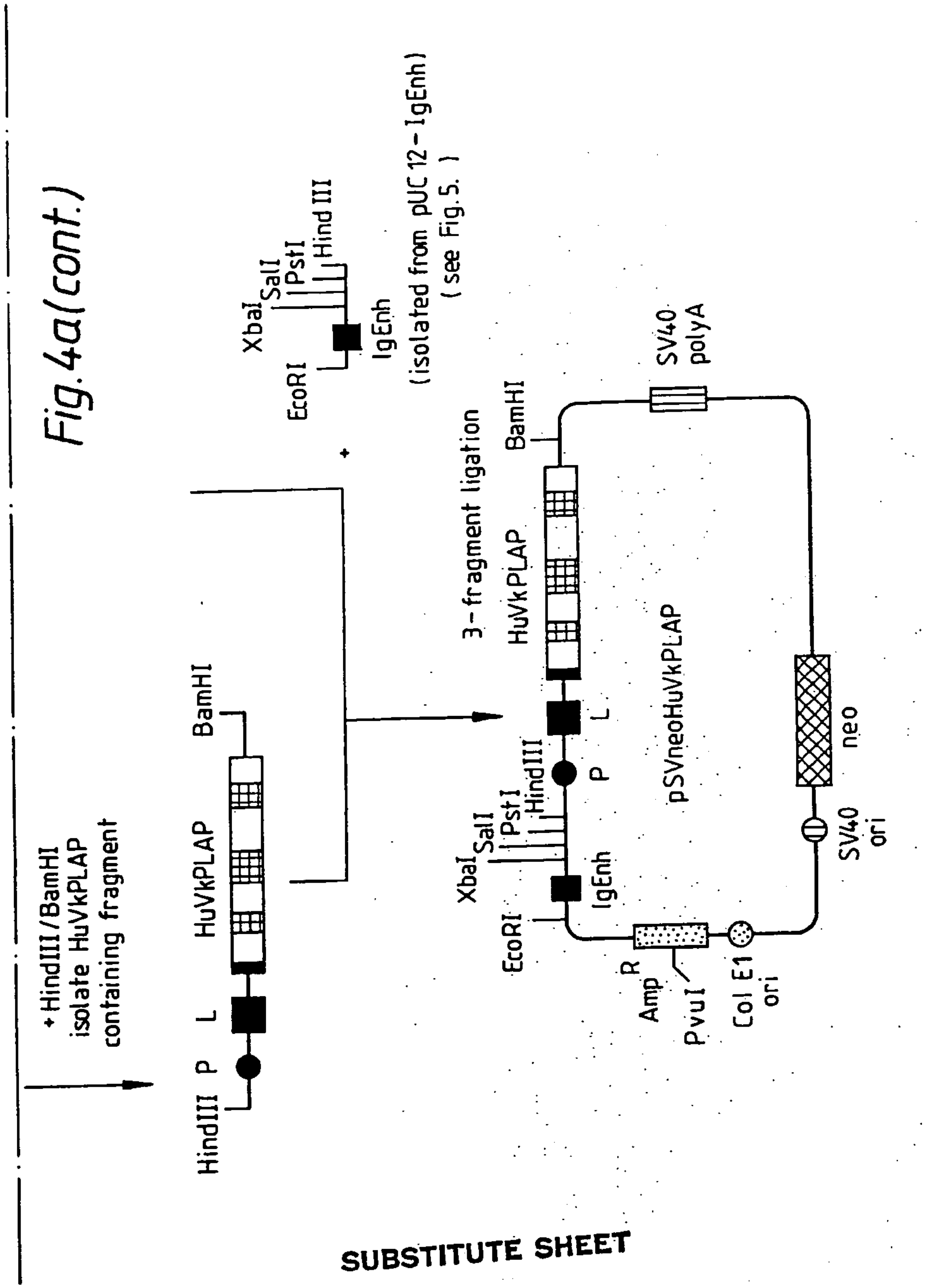

SV40
polyA
EcoRI BamHI
pSVneoMSN409
neo
SV40
ori
R
Amp
PvuI
Col E1
ori
+ EcoRI/BamHI
isolate large vector
fragment
HindIII P L VI VII VIII BamHI
HuVkLYS
M 13mp 9 HuVkLYS
CDR graft by site directed
mutagenesis
HindIII P L HuVkPLAP BamHI
M 13mp 9 HuVkPLAP

Fig.4a

Fig.4a(cont.)


+HindIII/BamHI
isolate HuVkPLAP
containing fragment
HindIII
P
L
HuVkPLAP
BamHI
EcoRI
XbaI
SalI
PstI
Hind III
IgEnh
(isolated from pUC 12 - IgEnh)
(see Fig.5.)
3-fragment ligation
BamHI
SV40
polyA
HuVkPLAP
pSVneoHuVkPLAP
L
P
HindIII
PstI
SalI
XbaI
IgEnh
EcoRI
neo
SV40
ori
Amp
R
PvuI
Col E1
ori

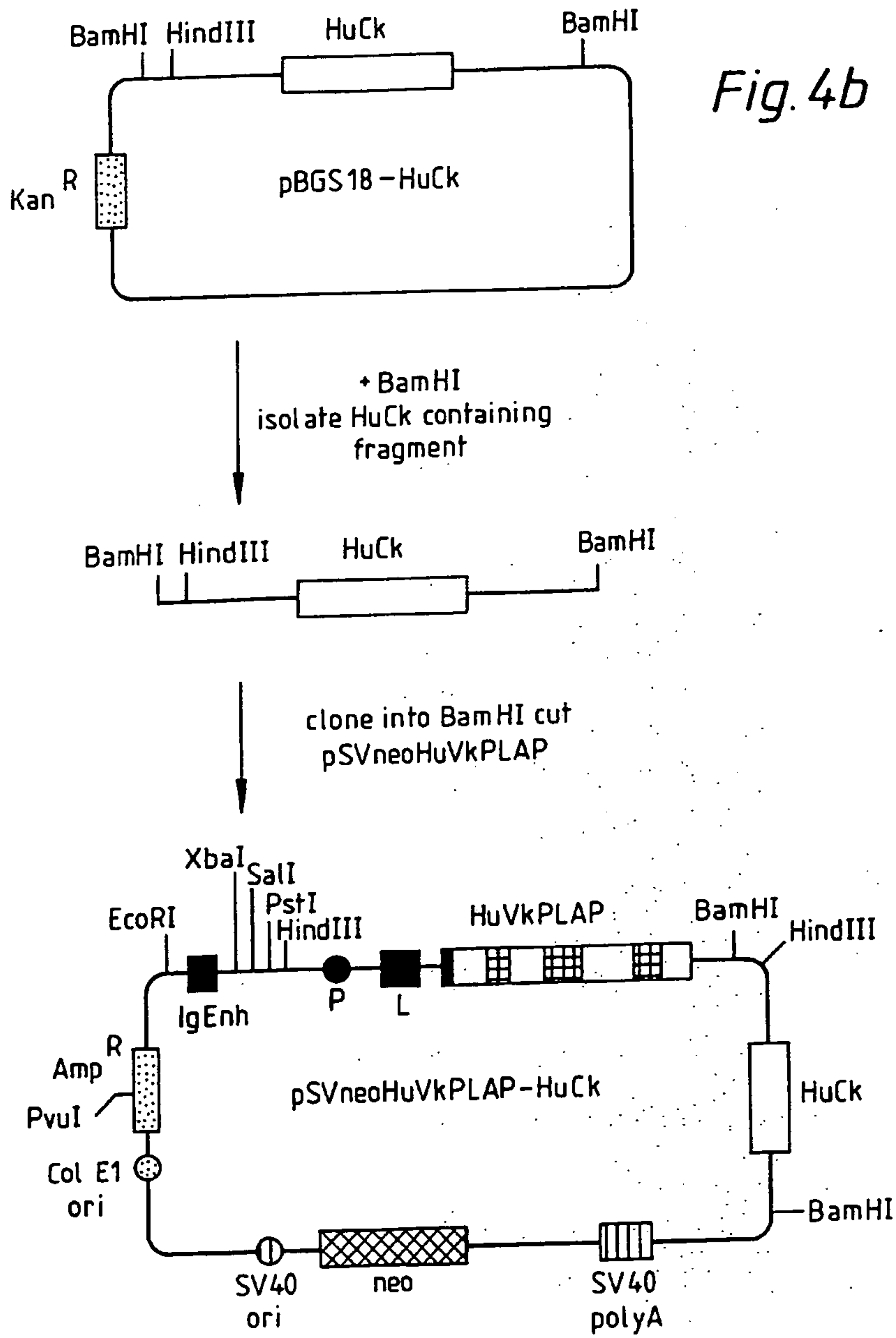

BamHI
HindIII
HuCk
BamHI
Kan R
pBGS18-HuCk
+ BamHI
isolate HuCk containing fragment
BamHI
HindIII
HuCk
BamHI
clone into BamHI cut pSVneoHuVkPLAP
XbaI
SalI
PstI
HindIII
EcoRI
HuVkPLAP
BamHI
HindIII
IgEnh
P
L
Amp R
PvuI
pSVneoHuVkPLAP-HuCk
HuCk
Col E1 ori
BamHI
SV40 ori
neo
SV40 polyA

Fig. 4b

Fig. 5.

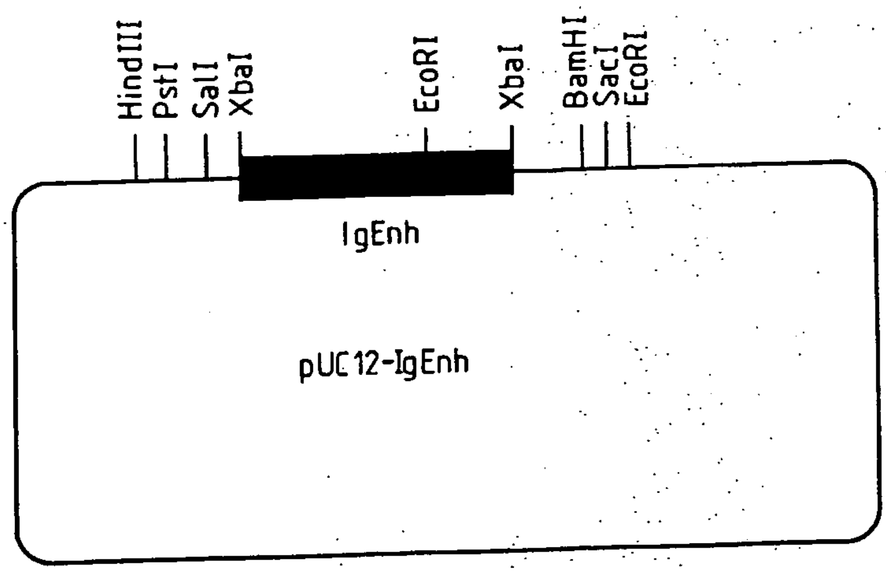

HindIII
PstI
SalI
XbaI
EcoRI
XbaI
BamHI
SacI
EcoRI
IgEnh
pUC12-IgEnh

Fig. 6.

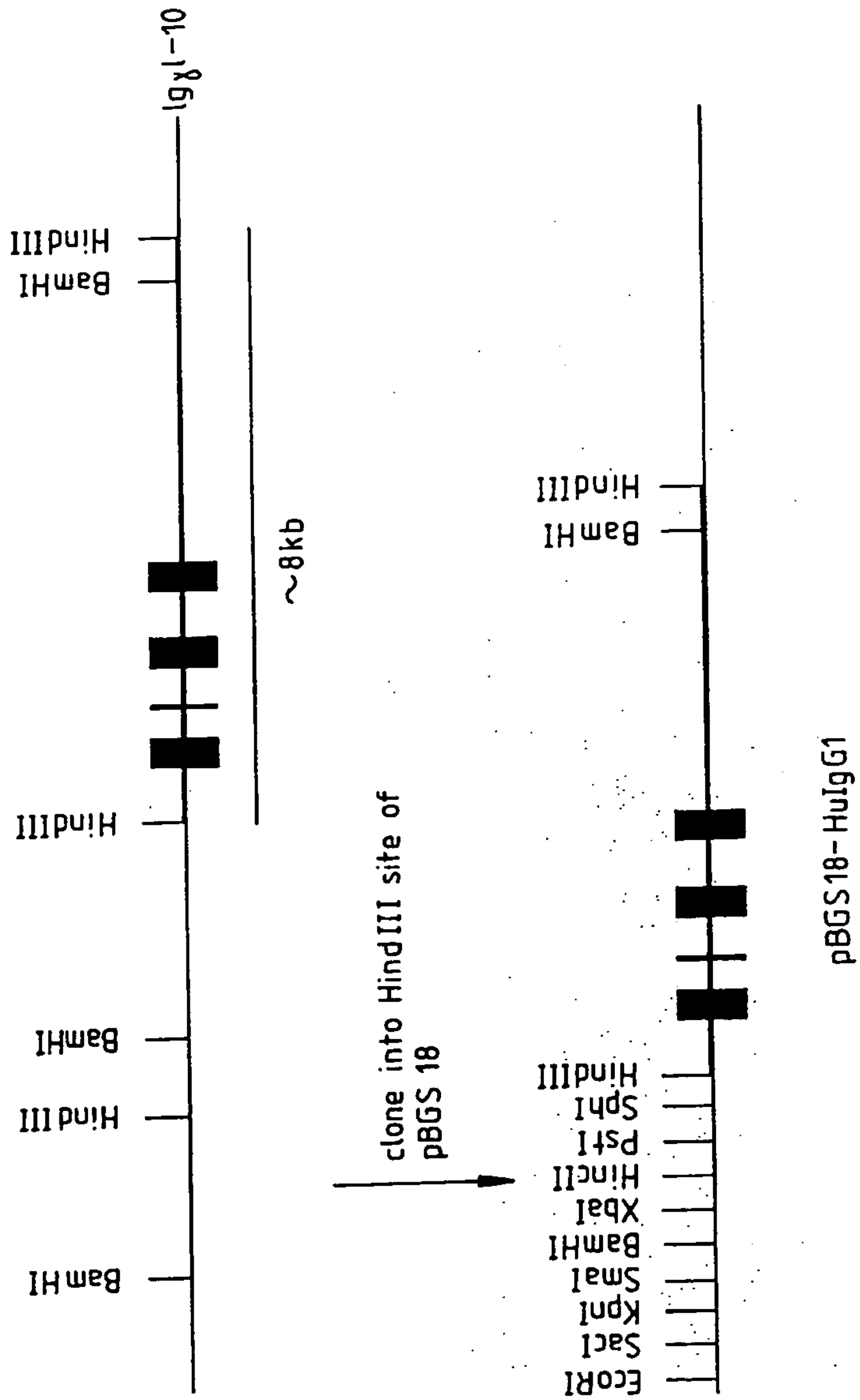
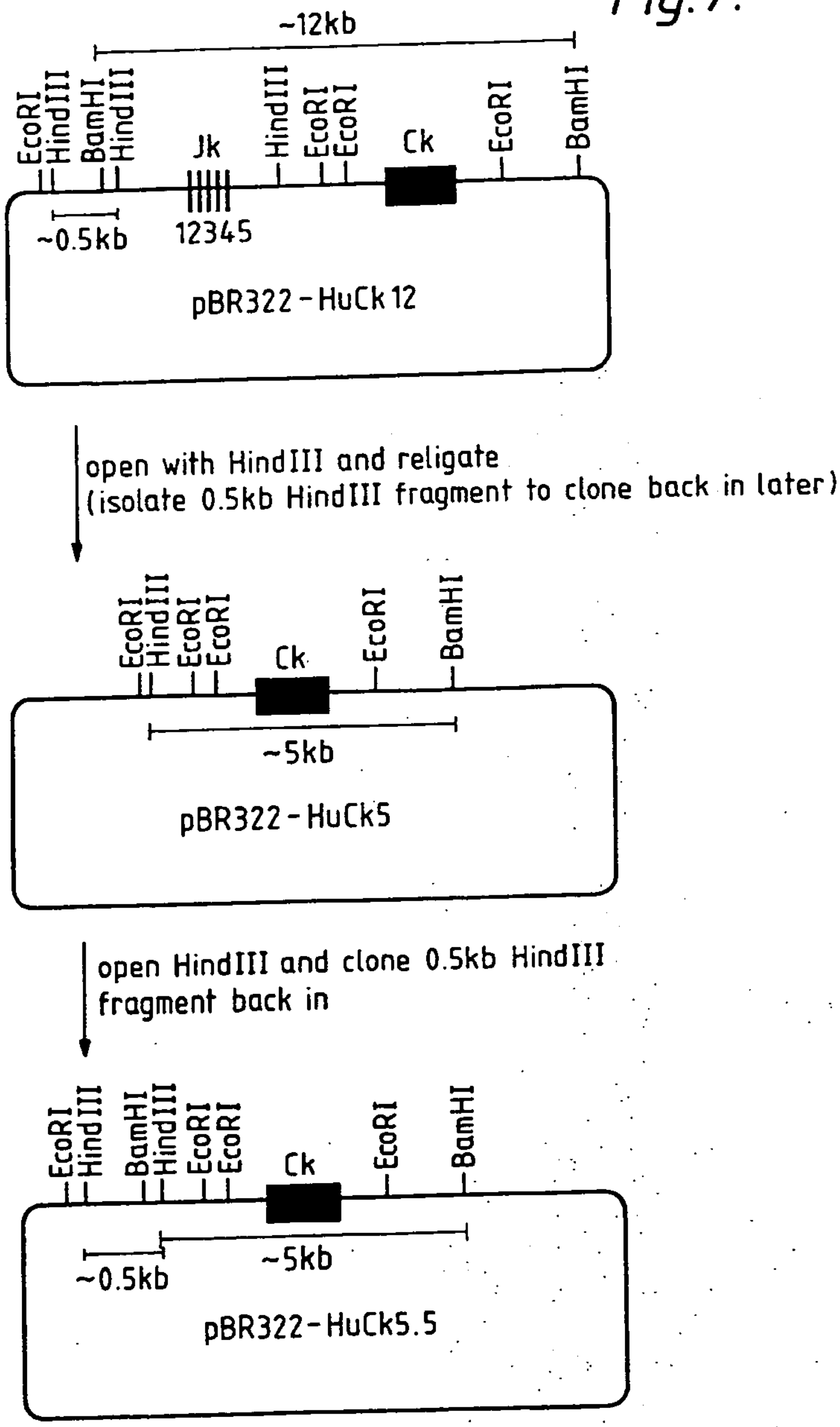

Igγ1-10
HindIII
BamHI
~8kb
HindIII
BamHI
HindIII
BamHI
clone into HindIII site of
pBGS 18
HindIII
BamHI
pBGS18-HuIgG1
HindIII
SphI
PstI
HincII
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI

Fig. 7.



Subclone HuCk containing BamHI fragment in pBGS18-BamHI gives: pBGS18-HuCk

SUBSTITUTE SHEET

Fig. 8.

Oligonucleotides used for cloning variable region genes

I : mouse constant gamma1 primer

5' GAT AGA CAG ATG GGG GTG TCG TTT 3'

II : mouse constant kappa primer

5' AGA TGG ATA CAG TTG GTG CAG CAT 3'

Fig. 9.

<u>Oligonucleotides used for CDR grafting</u>

III : VHPLAP-CDR1

5' CTG TCT CAC CCA <u>GCT TAC ACC ATA ACT</u> *GGT* GAA GGT *GAA* GCC

AGA CAC GGT 3'

IV : VHPLAP-CDR2

5' CAT TGT CAC TCT <u>GGA TAT GAG AGC TGA ATG ATA ATT TGT GCT</u>

<u>CCC GTC TTC CCA TAT TAC</u> TCC AAT CCA CTC 3'

V : VHPLAP-CDR3

5' GCC TTG ACC CCA <u>GTA TTC CAT AGC CCC CAC GTA GCT GCT ACC</u>

<u>GTA GTG GGG</u> TCT TGC ACA ATA 3'

Fig.9(cont.)

VI : VkPLAP-CDR1

5' CTG CTG GTA CCA TGC TAC ATA ACT GTA AAT ATT TTC ACT TGC

TCG ACA GGT GAT GGT 3'

VII : VkPLAP-CDR2

5' GCT TGG CAC ACC CTC TGC TAA GGA TTT TGC ATT GTA GAT CAG

CAG 3'

VIII : VkPLAP-CDR3

5' CCC TTG GCC GAA CGT CCA CGG ACT AAC ATA ATG ATG TTG

GCA GTA GTA GGT 3'

Fig. 10.

Hu2VHPLAP

```
                5                   10                  15                  20
CAG GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG ACC CTG AGC CTG    60
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln Thr Leu Ser Leu

                25                  30    CDR 1     35                  40
ACC TGC ACC GTG TCT GGC TTC ACC TTC ACC AGT TAT GGT GTA AGC TGG GTG AGA CAG CCA   120
Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Ser Tyr Gly Val Ser Trp Val Arg Gln Pro

                45                  50                  55    CDR 2     60
CCT GGA CGA GGT CTT GAG TGG ATT GGA GTA ATA TGG GAA GAC GGG AGC ACA AAT TAT CAT   180
Pro Gly Arg Gly Leu Glu Trp Ile Gly Val Ile Trp Glu Asp Gly Ser Thr Asn Tyr His

                65                  70                  75                  80
TCA GCT CTC ATA TCC AGA GTG ACA ATG CTG GTA GAC ACC AGC AAG AAC CAG TTC AGC CTG   240
Ser Ala Leu Ile Ser Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu

    82  A   B   C   83      85                  90                  95    CDR 3
AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC TAT TAT TGT GCA AGA CCC CAC TAC   300
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Pro His Tyr

        100 A   B   C   D   E   101             105                 110
GGT AGC AGC TAC GTG GGG GCT ATG GAA TAC TGG GGT CAA GGC AGC CTC GTC ACA GTC TCC   360
Gly Ser Ser Tyr Val Gly Ala Met Glu Tyr Trp Gly Gln Gly Ser Leu Val Thr Val Ser

TCA                                                                                363
Ser
```

Fig.11.

HuVkPLAP

```
                5                   10                  15                  20
GAC ATC CAG ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT GAC AGA GTG ACC    60
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr
                25      CDR 1       30                  35                  40
ATC ACC TGT[CGA GCA AGT GAA AAT ATT TAC AGT TAT GTA GCA]TGG TAC CAG CAG AAG CCA   120
Ile Thr Cys[Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Val Ala]Trp Tyr Gln Gln Lys Pro
                45                  50      CDR 2       55                  60
GGT AAG GCT CCA AAG CTG CTG ATC TAC[AAT GCA AAA TCC TTA GCA GAG]GGT GTG CCA AGC   180
Gly Lys Ala Pro Lys Leu Leu Ile Tyr[Asn Ala Lys Ser Leu Ala Glu]Gly Val Pro Ser
                65                  70                  75                  80
AGA TTC AGC GGT AGC GGT AGC GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC CTC CAG CCA   240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
                85                  90      CDR 3       95                  100
GAG GAC ATC GCC ACC TAC TAC TGC[CAA CAT CAT TAT GTT AGT CCG TGG ACG]TTC GGC CAA   300
Glu Asp Ile Ala Thr Tyr Tyr Cys[Gln His His Tyr Val Ser Pro Trp Thr]Phe Gly Gln
                105
GGG ACC AAG GTG GAA ATC AAA CGT                                                   324
Gly Thr Lys Val Glu Ile Lys Arg
```

Fig. 12.

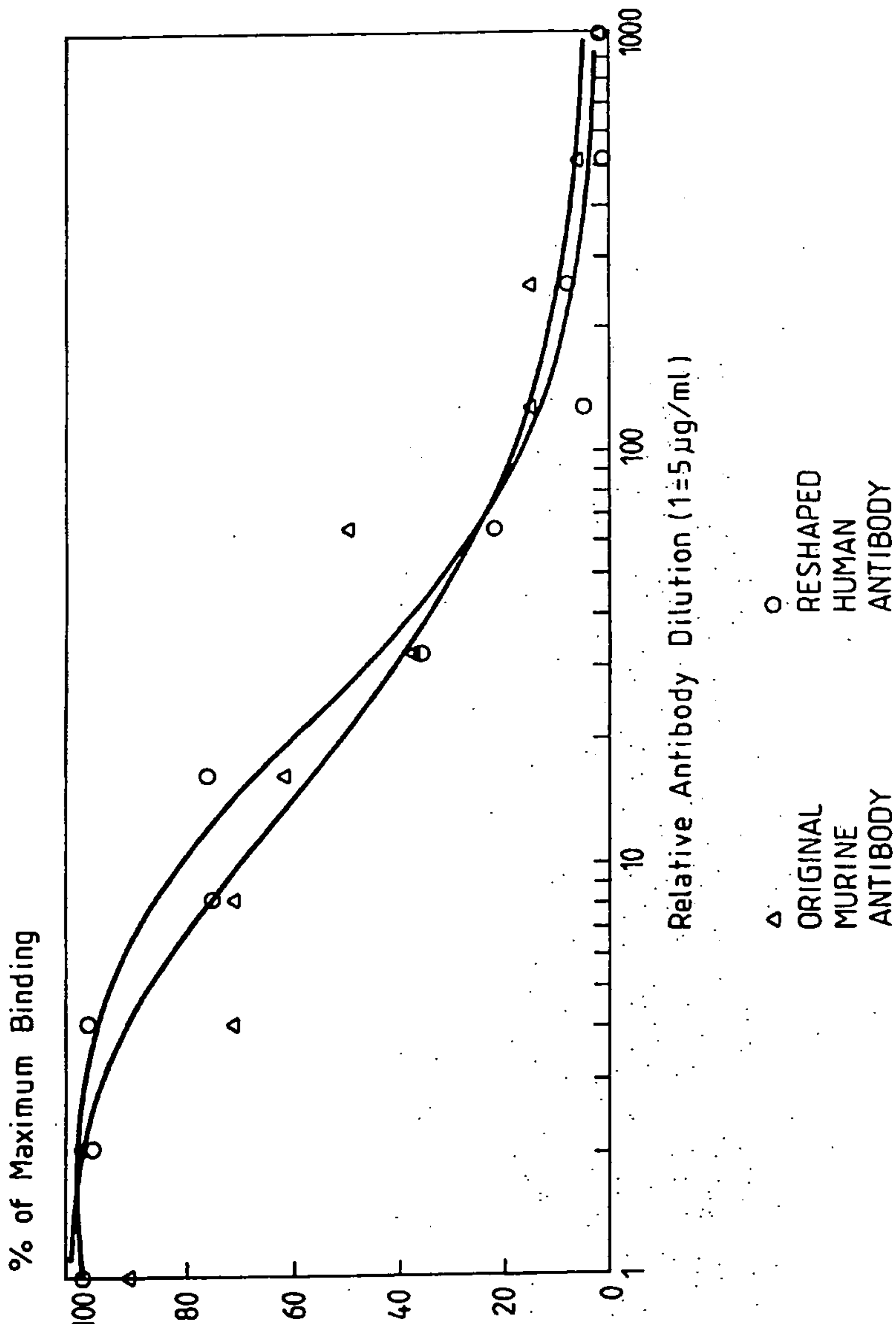
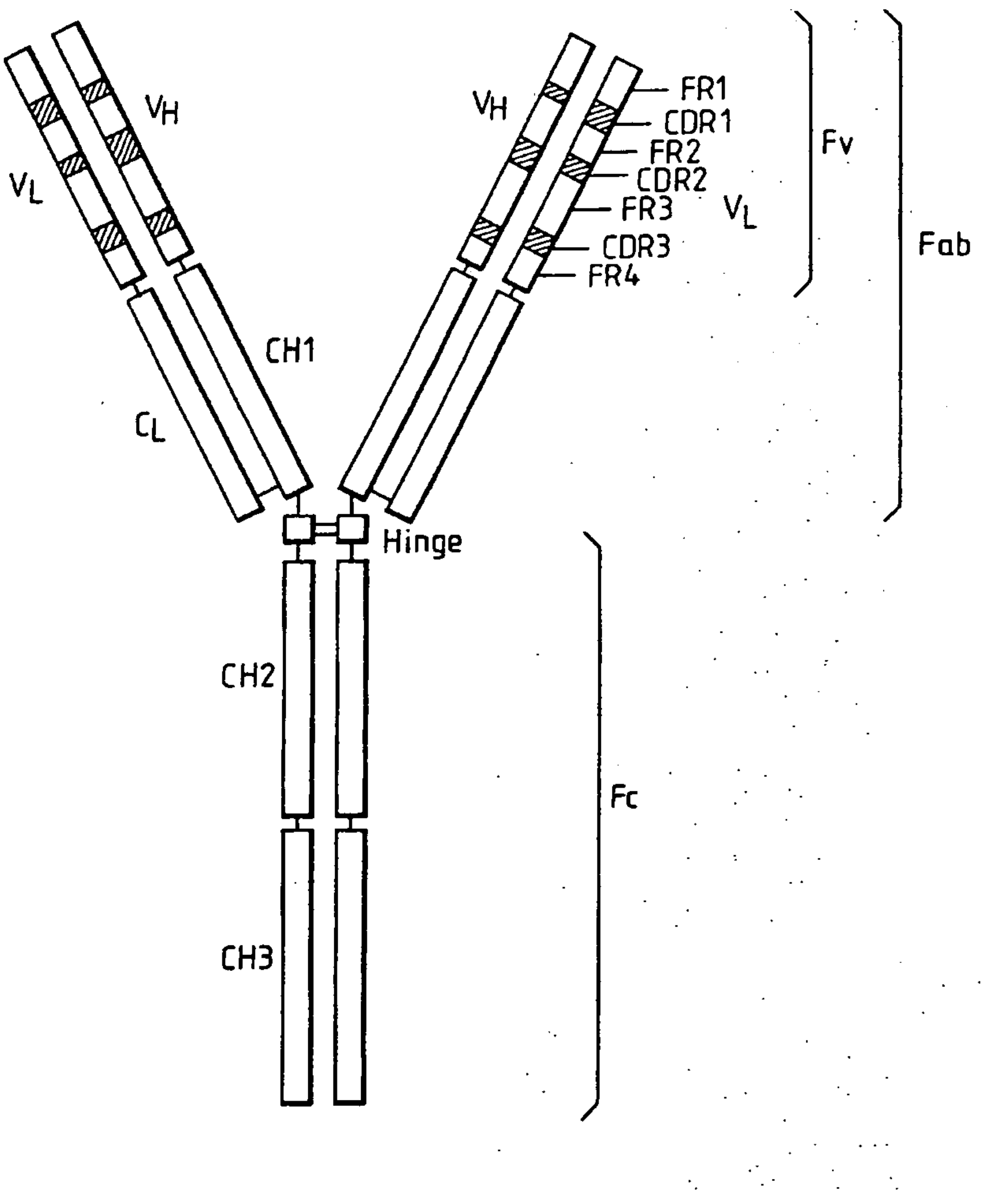

% of Maximum Binding
100
80
60
40
20
0
1
10
100
1000
Relative Antibody Dilution (1=5 μg/ml)
ORIGINAL MURINE ANTIBODY
RESHAPED HUMAN ANTIBODY

Fig.13.


VL
VH
CL
CH1
VH
FR1
CDR1
FR2
CDR2
FR3
VL
CDR3
FR4
Fv
Fab
Hinge
CH2
CH3
Fc

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/01755

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC C 12 P 21/08,
IPC^5: C 12 N 15/13, C 12 N 5/10, A 61 K 39/395, G 01 N 33/574

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC^5	C 12 N, C 12 P, C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	European Journal of Biochemistry, volume 176, no. 2, 1988, P. De Waele et al.: "Expression in non-lymphoid cells of mouse recombinant immunoglobulin directed against the tumour marker human placental alkaline phosphatase", pages 287-295 see the whole article	1,2,11, 28-30
Y		3-10,12-27
Y	EP, A, 0239400 (G.P. WINTER) 30 September 1987 see the whole document cited in the application	3-10,12-27

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11th March 1991	10 APR 1991
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MISS D.S. KOVALCIK

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 31 because they relate to subject matter not required to be searched by this Authority, namely:

Pls. see Rule 39.1 (iv) - PCT:

Method for treatment of the human or animal body by therapy or surgery , as well as diagnostic method.

2. [ ] Claim numbers ...., because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers ...., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9001755
SA 42041

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 03/04/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0239400	30-09-87	GB-A,B 2188638 JP-A- 62296890	07-10-87 24-12-87

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82